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Antitumor Activities of Lipophilic Nucleoside 5′-monophosphate Analogues as Prodrugs

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Abstract

Several nucleoside 5′-monophosphate analogues and lipophilic nucleoside 5′-(3-pyridinylcarbonyl)monophosphate analogues were synthesized. Antitumor activities of the synthesized nucleoside analogues against *P388 mouse leukemia, FM3A murine mammary carcinoma,* and *U937 human histiocytic lymphoma* cells were determined by MTT assay. Antitumor activities of the lipophilic uridine 5′-(3-pyridinylcarbonyl)monophosphate**(7)** and 2′,3′-didehydro-3′-deoxy-thymidine-5′-(3-pyridinylcarbonyl)monophosphate**(8)** were stronger than those of uridine 5′-monophosphate**(1)** and 2′,3′-didehydro-3′-deoxythymidine-5′-monophosphate**(4)**. This preliminary experimental result suggests that nucleoside 5′-(3-pyridinylcarbonyl)monophosphate analogues may be new prodrugs to overcome the clinical limit.

Key words : *antitumor activity, lipophilic nucleoside analogue*

Introduction

Chemotherapeutic agent was known as chemical substance which acted directly to pathogen and killed it or depressed its growth and then cured the infection disease. It contained antiviral agents, antitumor agents, antibiotics, *et al* [4]. Hichings *et al.* had tried to modify the purine and pyrimidine component of nucleic acid 50 years ago [5, 14], and this research provoked many workers to develop the nucleoside analogues which were used in the removal of tumor and viral infectious disease. The reason for the active study of nucleoside analogue was because its physiological function was various [2, 15] and the cell membrane permeability was good [1, 6] and the possibility of structural modification was unlimited.

Although useful nucleoside analogues were studied and reached to the clinical step the toxicity and resistance for normal cell were pointed as the clinical limit. In the case of tumor and viral infection, the toxicity for normal cell was strong. Now we are trying to maintain the effect of the pre-developed drug and solve the above problems. The most promising study is the one on the lipophilic nucleoside monophosphate analogue [3, 7, 9-11]

Several nucleoside 5′-monophosphate and lipophilic nucleoside 5′-(3-pyridinylcarbonyl) monophosphate analogues were synthesized in this study [7] and then the antitumor activities [13] of the synthesized nucleoside analogues for tumor cells were determined.

Materials and Method

Reagents

Uracil, uridine, thymidine, phosphorus pentasulfide, 0.5M nitronium tetrafluoroboric acid sulfolan, methanesulfonyl chloride, nicotinic acid, and KOtBu were

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purchased from Aldrich Co(USA). Triethyl phosphate was from Fluka Co(Swiss). MTT(3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) was obtained from Sigma Co(USA).

Instruments

Melting point of each synthesized compound was measured using electrothermal capillary melting point measuring apparatus. ^{1}H-NMR spectrum was obtained on Varian EM-360A(60 MHz), Varian Gemini-200(200MHz), and Bruker AM 300(300MHz) spectrometer. ^{13}C-NMR spectrum was obtained on Bruker AM 300 spectrometer and IR spectrum on Bomen spectrometer using KBr pellet. ^{31}P-NMR spectrum was obtained from Pohang University of Science and Techonology. ELISA Processor II Microplate was used to measure absorbance in determining IC_{50}.

Synthetic procedure

All the solvents were used after purification according to general procedure [11]. The completion of reaction was identified by TLC. Silica gel for TLC was silica gel 60 F_{254}(Merck) and the reaction was identified using UV (254nm) or anisaldehyde solution [3]. Distillation was carried out with rotary evaporator under the vacuum below 30°C or with short-path distillation apparatus cooling on dryice-acetone.

Synthesis of nucleoside 5'-monophosphate analogue (Fig. 1)

$POCl_3$(7.42 mmol) was added to redistilled $(EtO)_3PO$ on BaO at -5 - 0°C and nucleoside analogue(4mmol) was reacted and stirred for 3h. Cool distilled water was slowly added on ice-bath and extracted triethylphosphate with diethyl ether, and then neutralized water layer to pH 7.0 with saturated NaCl solution. Water layer was vacuum dried to obtain white solid. This solid was dissolved in minimal amount of distilled water, separated with silica gel column(10mm x 20cm, $CHCl_3$: MeOH : H_2O=65:25:4), and the solvents were vacuum dried, washed with acetone, and recrystallized with EtOH to produce nucleoside 5'-monophosphate analogue.

Uridine 5'-monophosphate(1) : mp 208-210(dec.), ^{31}P-NMR (D_2O) δ 0.98, ^{1}H-NMR(D_2O) δ 8.15(d, 1H, H-6), 5.9-6.1(m, br, 3H), 4.85(s, 2H), 4.1(d, 1H), 4.5-4.3(m, 2H, H-5'), IR(KBr) 3100, 1610, 1250, 1080 and 1055 cm^{-1}.

2',3'-O-isopropylidene uridine 5'-monophosphate**(2)** : mp 177-180°C ^{1}H-NMR(D_2O) δ 7.77(d, 1H, H-5, 6), 5.79(t, 3H), 4.78(s, 2H), 4.24(s, 1H), 3.81(s, 2H, H-5'), 1.42 & 1.23,. IR(KBr) 3451, 1680, 1092 cm^{-1}

5-Nitrouridne 5'-monophosphate(3) : ^{1}H-NMR(D_2O) δ 7.01(s, 1H, H-6), 5.95(s, 1H, H-1'), 5.21(s, 2H, H-5'), 4.23 (d, 2H, H-2', H-3'), 2.06(s, 9H, $(CH_3)_3$), IR(KBr) 1650, 1700(amide C=O), 1400 cm^{-1}(-NO_2).

2',3'-didehydro-3'-deoxythymidine-5'-monophosphate (4) : mp 283- 285°C, ^{1}H-NMR(D_2O) δ 7.48(s, 1H, H-6), 6.75(s, 1H, H-1'), 6.34(d, 1H, H-3'), 5.79(dd, 1H, H-2'), 4.85(s, 1H, H-4'), 4.34(dd, 2H, H-5'), 1.70(s, 3H, CH_3), ^{13}C-NMR(D_2O) δ 157.03(C-4), 151.49(C-2), 148.55(C-6), 124.01 (C-3'), 86.98(C-2'), 86.37(C-5), 71.41(C-1'), 69.57 (C-4'), 62.64(C-5'), 12.32 (CH_3), IR(KBr) 3456, 1672, 1253, 1171, 1045, 975 cm^{-1}.

3-β-D-ribosyl-8-azaxanthine 5'-monophosphate(5) : ^{1}H-NMR (D_2O) δ 10.49(br, NH), 7.93(s), 7.27-7.19(br, 1H, H-1'), 6.15-6.12(s, s, 2H, H-5'), 5.11(m, 2H, H-2', H-3'), 4.90(m), IR(KBr) 3248, 1964, 1533, 1404, 1300, 1078 cm^{-1}.

2',3'-O-diacetyl uridine 5'-monophosphate(6) : mp 177-180°C, ^{1}H-NMR(D_2O) δ 9.48(d, 1H, H-6), 5.6-5.1(s, br, 3H), 3.01(s, 2H, H-5'), 2.2-1.9(s, $(CH_3)_2$), IR(KBr) 3400, 1720, 1657, 1610, 1080, and 1055 cm^{-1}.

Synthesis of nucleoside 5'-(3-pyridinylcarbonyl) monophosphate analogue(7, 8).

$POCl_3$(0.22mL, 2.4mmol) was added to redistilled $(EtO)_3PO$(10mL) on BaO at -5 - 0°C and dried nicotinic acid(0.246g, 2mmol) was reacted. Nucleoside analogue was added and stirred for 10h at -5 - 0°C. Cool distilled water was slowly added and extracted triethylphosphate with diethyl ether, and then neutralized water layer to pH 7.0. Water layer was vacuum dried to obtain white

solid. This solid was dissolved in minimal amount of distilled water, separated with silica gel column, and the solvents were vacuum dried, and recrystallized with EtOH to produce nucleoside 5′-(3-pyridinylcarbonyl) monophosphate analogue.

Uridine 5′-(3-pyridinylcarbonyl)monophosphate(7) : mp 188-190°C, ^{1}H-NMR(DMSO-d_6) δ 11.35(s, 1H, NH), 9.08 (s, 1H, pyridine H-2), 8.83(d, 1H, pyridine H-6), 8.31(d, 1H, pyridine H-4), 8.27(s, 1H, H-6), 7.65(m, 1H, pyridine H-5), 5.76(d, 1H, H-1′), 5.54(d, 1H, H-2′), 5.35(d, 1H, H-3′), 4.54(m, 1H, H-4′), 4.13(s, 1H, H-5′), 2.49(s, 3H, CH_3), ^{13}C-NMR (DMSO-d_6) δ 164.60(C-4), 163.03(pyridine C-2), 153.85(pyridine C-6), 150.56(C-2), 150.05(pyridine C-1), 140.99(carbonyl C), 136.98(C-6), 125.42 (C-3′), 123.99(C-2′), 101.93(C-5), 89.28(C-1′), 80.86(C-4′), 72.71(pyridine C-4), 69.67(pyridine C-5), 64.61(C-5′), IR(KBr) 3434, 3106, 1696, 1444, 1270, 1091 cm^{-1}.

2′,3′-didehydro-3′-deoxythymidine 5′-(3-pyridinylcarbonyl) monophosphate(8) : mp 221-223°C(slowly dec), ^{1}H-NMR(DMSO-d_6) δ 11.35(s, 1H, NH), 9.08(s, 1H, pyridine H-2), 8.83(d, 1H, pyridine H-6), 8.31(d, 1H, pyridine H-4), 8.27(s, 1H, H-6), 7.65(m, 1H, pyridine H-5), 5.76(d, 1H, H-1′), 5.54(d, 1H, H-2′), 5.35(d, 1H, H-3′), 4.54(m, 1H, H-4′), 4.13(s, 1H, H-5′), 2.49(s, 3H, CH_3), ^{13}C-NMR(DMSO-d_6) δ 164.60(C-4), 163.03(pyridine C-2), 153.85(pyridine C-6), 150.56(C-2), 150.05(pyridine C-1), 140.99(carbonyl C), 136.98(C-6), 125.42(C-3′), 123.99(C-2′), 101.93 (C-5), 89.28(C-1′), 80.86(C-4′), 72.71(pyridine C-4), 69.67(pyridine C-5), 64.61 (C-5′), 12.01(CH_3), IR(KBr) 3434, 3106, 1696, 1444, 1270, 1091 cm^{-1}.

Antitumor activity assay

MTT was dissolved in PBS(phosphate buffered saline, pH 7.4) to 5mg/mL, filtered, and stored in dark room at 4°C. Various concentrations of test compounds were added to *P388, FM3A,* and *U937* cells($2x10^4$ cells/well) in Dulbecco's modified Egele's medium. The upper 160μL medium was removed from the incubated microtitered well and 10-diluted 100μL MTT solution with serum-free RPMI 1640 medium was added to each well. The well was incubated for 4h at 37°C, 5% CO_2 and carefully removed the upper solution. 100μL DMSO was added to each well and the resultant formazon was dissolved by mixing with plate shaker for 20-30 min. The absorbances at 570 and 650nm were measured with ELISA Processor II Microplate reader.

Results and Discussion

Syntheses of nucleoside 5′-monophosphate and nucleoside 5′-(3-pyridinylcarbonyl)monophosphate analogues

Nucleoside 5′-monophosphate and nucleoside 5′-(3-pyridinyl carbonyl) monophosphate analogues were synthesized as the Hong *et al*'s method. The synthetic reaction of nucleoside 5′-(3-pyridinylcarbonyl) monophosphate analogues was one step reaction which added nucleoside analogues to 3-pyridinylcarbonyl dichloride phosphate intermediate formed in the reaction of nicotinic acid and $POCl_3$ under the $(EtO)_3PO$ solvent without coupling reagent and enzyme. Since it was acidic condition and exothermic reaction, acid-catalyzed hydrolysis of acid and sugar was occurred [13]. Therefore this reaction was required to terminate within short time and a special attention was paid in the neutralization process. Syntheses of nucleoside analogues were identified by the specific peaks on ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR spectrum and the strong P=O peaks on IR spectrum at the 1094, 1091 cm^{-1} positions.

Antitumor activities of synthesized nucleoside analogues

The antitumor activities of the synthesized nucleoside derivatives for tumor cells were shown in Table 1. All nucleoside derivatives were shown inhibition more than 80% for *U937* cell and 50 and 60% for *FM3A* and *P388* cells, respectively. The percentage inhibition of uridine 5′-(3-pyridinylcarbonyl)monophosphate(7) was greater than uridine 5′-monophosphate(1) for three cell lines. Also

Table 1. Antitumor activity of nucleoside derivative(% inhibition at 100μg/ml[a]) against three tumor cells

Compound	Cell Line		
	FM3A[b]	P388[c]	U937[d]
Uridine 5'-monophosphate(1)	50<	50<	50<
2',3'-didehydro-3'-deoxy-thymidine 5'-monophosphate(4)	67	64	>85
Uridine 5'-(3-pyridinyl carbonyl)monophosphate(7)	52	61	80
2',3'-didehydro-3'-deoxy-thymidine 5'-(3-pyridinyl carbonyl)monophosphate(8)	71	69	>90

a : Determined by MTT assay described in ref. 13.
b : *Murine mammary carcinoma FM3A* cell.
c : *Mouse leukemia P388* cell.
d : *Human histiocytic lymphoma U937* cell.

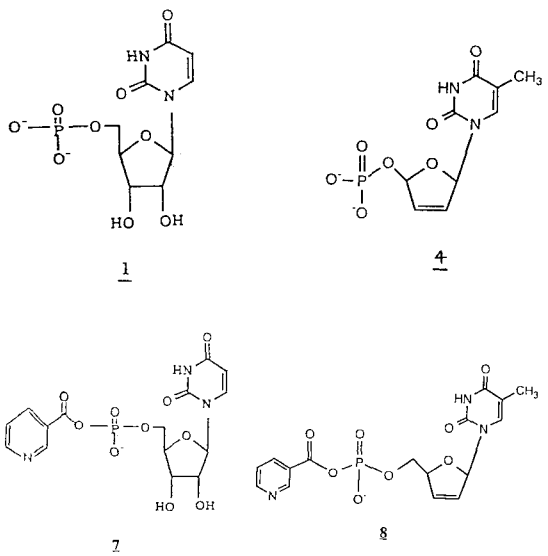

Fig. 1. Nucleoside 5'-monophosphate analogues

2',3'-didehydro-3'-deoxythymidine-5'-(3-pyridinylcarbonyl) monophosphate (8) showed stronger inhibition than 2',3'-didehydro-3'-deoxythymidine-5'-monophosphate(4) for *FM3A* cell.

Most biologically active nucleoside was phosphorylated as mono-, di-, or triphosphate in order to combine to RNA or DNA by the kinase of host before showing inhibitory effect for a specific enzyme [8]. The rate determining step in the absorption of nucleoside was the first phosphorylation step, i.e. the formation step of nucleoside 5'-monophosphate analogue [14].

When nucleoside 5'-monophosphate analogues passed through cell membrane by active diffusion [1, 6] the two negative charges of phosphate made it difficult to pass through the cell membrane, so an effective transport pathway was needed. Simple structural nicotinic acid which was a component of vitamin was esterificated to mask negative charge and increase the lipophilicity, and then to make active diffusion easy in this study. When nucleoside 5'-(3-pyridinylcarbonyl)monophosphate analogues entered into target cell, it was hydrolyzed to nucleoside or nucleoside monophosphate by phosphodiesterase, so the first phosphorylation process, was able to be omitted [12].

This result suggested that nucleoside 5'-(3-pyridinylcarbonyl)monophosphate analogues might be a solution in the removal of pathogens which had resistance to pre-nucleoside chemotherapeutic agent and in the treatment of chemotherapeutic agent which had a toxicity for normal cell because of its abnormal phosphorylation. Furthermore, when other drugs except nicotinic acid were esterificated, the synergistic effect was expected and then the development of a new chemotherapeutic agent which had better cure would be come true.

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초록 : Prodrug로서 지질친화성 Nucleoside 5′-(3-pyridinyl carbonyl) monophosphate 유도체의 항암 활성

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몇가지 nucleoside 5′-monophosphate 유도체들과 지질 친화성을 증가시킨 nucleoside 5′-(3-pyridinyl carbonyl)monophosphate 유도체들을 합성한 후 *Mouse leukemia P388, Murine mammary carcinoma FM3A, Human histiocytic lymphoma U937* 세포들에 대해 시험관내에서 항암활성을 MTT를 이용한 방법으로 나타내었다. 그 결과 uridine 5′-(3-pyridinylcarbonyl) monophosphate(7)와 2′,3′-didehydro-3′-deoxythymidine-5′-(3-pyridinylcarbonyl) monophosphate(8)의 inhibition이 uridine 5′-monophosphate(1)와 2′,3′-didehydro-3′-deoxythymidine-5′-monophosphate(4) 보다 각각 증가하였다. 이는 nucleoside 5′-(3-pyridinylcarbonyl) monophosphate 유도체들이 임상적 한계를 극복할 수 있는 가능성을 보인 것이다.

주제어 : prodrug, nucleoside monophosphate 유도체, 항암 활성